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Abstract

Remote ischemic preconditioning (RIPC) is a non-invasive technique in which blood flow is cut off to a limb for a short period of time which produces a protective effect throughout the whole body to subsequent low oxygen conditions. One of the prominent cardiovascular limitations to exercise in individuals with multiple sclerosis (MS) is fatigue, which may stem from the decreased delivery of oxygen to muscle tissue during exercise. The purpose of this study was to test the hypothesis that RIPC would increase forearm blood flow (FBF) and utilization of oxygen at the level of the muscle tissue in individuals with MS during handgrip exercise. FBF and forearm vascular conductance (FVC) were measured using Doppler Ultrasound technology during rhythmic handgrip exercise both before and after the RIPC intervention, with muscle oxygen saturation and system hemodynamics measured throughout the protocol. The results of the present pilot study did not indicate an effect of RIPC on exercise hyperemia; however, due to the small sample size, further study is worthwhile in order to more accurately determine if RIPC may serve to increase exercise capacity and quality of life for individuals with MS.

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Introduction

Multiple Sclerosis (MS) is an autoimmune disease affecting approximately 2.3 million people worldwide. It is characterized by demyelination and inflammation of the central nervous system (CNS) and symptoms vary among individuals depending on the location of the affected tissue. Currently, the exact etiology is unknown and while there is currently no cure for MS, treatment options include pharmacology aimed at reducing specific symptoms and managing relapses and rehabilitation to maintain and restore function (National Multiple Sclerosis Society). It is well-known that exercise provides numerous health benefits; however, MS presents several challenges to exercise including autonomic dysfunction, overheating, and fatigue (Cohen *et al.*, 1989; Bol *et al.*, 2012).

Impairment in autonomic nervous system (ANS) functioning, particularly the sympathetic nervous system (SNS), in patients with MS has been shown to serve as a limitation to exercise capacity due to its impact on fatigue. Decreased muscle sympathetic nerve activity (MSNA) and plasma norepinephrine (NE) concentrations (Keller *et al.*, 2014) along with reduced forearm blood flow (FBF) (Ranadive *et al.*, 2012; Huang *et al.*, 2015) in individuals with MS compared to healthy controls suggests impaired skeletal muscle circulation (Huang *et al.*, 2015). It has been consistently shown across several studies that patients with MS have a lower arterial blood pressure (BP) during both isometric and dynamic exercise (Huang *et al.*, 2015), suggesting an impaired pressor response. Decreased skeletal muscle circulation and attenuated BP responses would have a negative impact on exercise performance, along with limited muscle perfusion in patients with MS (Hansen *et al.*, 2013), because less oxygen would be available to be utilized to produce ATP in aerobic metabolism. Therefore, adequate blood flow to skeletal muscle is critical to reduce and delay fatigue.

There is evidence to suggest that remote ischemic preconditioning (RIPC) may benefit exercise performance in skeletal muscle. RIPC involves the application of brief ischemia to a peripheral tissue, such as a limb, to produce a protective response in other organs and tissues throughout the body (Przyklenk *et al.*, 1993). While the effects of RIPC have been most extensively studied on the heart, research suggests that RIPC is beneficial to improved exercise performance. RIPC has been observed to improve blood flow and decrease fatigue in skeletal muscle, therefore increasing exercise capacity

among healthy individuals (Wang *et al.*, 2004; Barbosa *et al.*, 2015). Little research has been done to date examining the effects of RIPC among individuals with MS. One recent review examined the mechanistic link between MS and RIPC in the pathophysiology of ischemic mechanisms and RIPC protection against inflammatory demyelination (Camara-Lemarroy *et al.,* 2018). In addition, it has been shown that RIPC offers promising implications on exercise tolerance among patients with MS based on a feasible double-blind RCT (Nair *et al.,* 2018).

It is suspected that RIPC will increase exercise hyperemia, the increase in blood flow that follows a muscle contraction to allow for greater oxygen delivery to skeletal muscle tissue, in individuals with MS. The purpose of this study is to test the hypothesis that RIPC will increase blood flow and utilization of oxygen at the level of the muscle tissue in individuals with MS during handgrip exercise.

Methods

Subjects

Eight volunteers, four healthy controls (35.8 ± 17 years) and four with MS (52.3 ± 7 years), provided written informed consent and underwent screening to determine eligibility. Inclusion criteria for those with MS included a clinical diagnosis of relapsing-remitting MS (RRMS), Expanded Disability Status Scale (EDSS) score between 0 and 4.5, and no relapse within 3 months of the study (Escudero-Uribe *et al.*, 2017) determined through the volunteers' self-report. The screening for all participants also included an assessment for body composition (within normal range according to ACSM guidelines) via bioelectric impedance (DC-430U, Tanita, Arlington Heights, IL), as well as ensuring that subjects were non-obese (BMI <25 kg/m^2), not hypertensive (resting blood pressure $<140/90$ mmHg), mild to moderately active as assessed by subjects' self-report, not pregnant, and not currently taking medication with significant cardiovascular effects. MS patients were allowed to continue their normal regimen, which most often included going to work and light physical activity throughout the day.

Rhythmic Handgrip Exercise

Subjects' maximal voluntary contraction (MVC) was determined by the average of three maximal squeezes of a handgrip dynamometer that were within 3% of one another. That average was then used to calculate the relative intensity workload of 10% MVC. A pulley system was utilized in which the subjects squeezed a handle approximately 3-4 cm to lift a weight corresponding to their calculated relative workload. The weight was lifted in a 1:2 sec duty cycle with visual and auditory feedback to ensure correct timing. The rhythmic handgrip exercise was performed for 4 minutes, with the 4th minute used to average local and systemic hemodynamics.

System Hemodynamics

Heart rate and beat-to-beat blood pressure were monitored via 3-lead ECG and finger photoplethysmography, respectively (Nova, Finometer). The finger pressure was calibrated to a brachial pressure obtained via automatic arm cuff sphygmomanometry.

Forearm Blood Flow (FBF) and Forearm Vascular Conductance (FVC)

FBF and FVC were measured using Doppler Ultrasound technology. A 12-MHz linear-array ultrasound probe (Vivid 8; General Electric, Milwaukee, WI) was utilized to determine brachial artery mean blood velocity (MBV) and brachial artery diameter. For blood velocity measurements, the probe insonation angle was maintained at $<60°$ and the frequency was 5 MHz. Brachial artery diameter measurements were mean in duplex mode at end-diastole and during steady-state conditions in triplicate. FBF was calculated as $FBF = MBV \times \pi$ (brachial artery diameter/2)$^2 \times 60$ where the FBF is in ml/min, the MBV is in cm/s, the brachial diameter is in cm, and 60 was used to convert from ml/s to ml/min. FVC was calculated as $FVC = (FBF/MAP) \times 100$ and is expressed as ml/min/100 mmHg (Crecelius *et al.*, 2011).

Muscle Oxygen Saturation (SmO$_2$) and Temperature

SmO$_2$ was measured using near-infrared non-invasive technology (Moxy Monitor, Moxy, Hutchinson, MN). The device was placed on the skin superficial to the extensor carpi radialis muscle (palpated during grip) and secured using a lightshield and tape. Temperature was measured using a probe placed on the skin just proximal to the muscle oxygenation sensor. Both remained in place the duration of the protocol.

Remote Ischemic Preconditioning

Blood pressure cuffs were wrapped around the subjects' right thigh, just proximal to the knee and rapidly inflated to 200 mmHg for 5 minutes of occlusion and deflated for 5 minutes of reperfusion. This procedure was repeated three times (for a total of 4 ischemic bouts) (de Groot *et al.*, 2010).

Experimental Protocol

Prior to the experimental session, the subjects followed a 4-hour fast and a 12-hour abstention from caffeine, alcohol, and exercise. The study took place in a cool (20-22°C) environment. After obtaining body composition and MVC measures, the subjects laid in a supine position with both arms abducted to 90°.

Following 20 minutes of quiet rest and instrumentation, subjects performed the rhythmic handgrip exercise (Figure 1). In addition, as part of a secondary study/protocol within the same subjects (Goetz Thesis), after 20 minutes of rest, subjects also performed a measure of vascular function via reactive hyperemia. Following both measures, the RIPC took place. The handgrip exercise and reactive hyperemia were both repeated following the RIPC, with 20 minutes of rest in between each measure.

Results

System Hemodynamics

As anticipated, HR increased for both control and MS subjects following a period of handgrip exercise; however, mean HR values were higher in the subjects with MS compared to the control subjects at all recorded time points. The mean MAP responses were increased with exercise among all participants with no clear difference between the two groups. RIPC did not seem to influence MAP values for either group. Mean skin temperature values remained consistent within the two groups regardless of exercise or RIPC condition; however, the skin temperature values were lower in subjects with MS compared to control subjects as expected (Table 2).

Forearm Blood Flow and Forearm Vascular Conductance

During rest, RIPC did not have an effect on FBF (Fig 2a) nor FVC (Fig 3a) as the changes were slight and inconsistent among subjects. With the exception of an outlier with significantly higher FBF and FVC values, the subjects with MS had lower FBF and FVC values compared to the control subjects as expected; however, the response to RIPC mirrored that of the control subjects.   Similarly to rest, during exercise RIPC slightly increased FBF (Fig 2b) and FVC (Fig 3b) for most subjects while decreasing for others. Therefore, RIPC does not appear to have had a large effect on either resting nor exercising FBF or FVC values, and no clear differences were seen between the MS and control subjects' responses to RIPC.

Muscle Oxygen Saturation

Resting SmO_2 percentages increased following RIPC for most subjects, with the MS subject's resting SmO_2 percentage increasing the greatest compared to the control (Fig 4a). RIPC did not appear to effect SmO_2 percentages during exercise, as some it remained relatively unchanged for most subjects (Fig 4b).

Discussion

The present study focused on the effects of RIPC on exercise capacity within healthy and MS populations. While the results are preliminary as it is a pilot study, RIPC did not appear to have a significant effect on exercise hyperemia specifically within the MS population. RIPC did not elicit greater FBF and FVC increases during exercise in subjects with MS nor the control subjects as hypothesized. In addition, SmO_2 was greater following the RIPC intervention during rest, with subjects with MS showing a greater increase compared to the control. This indicates increased O_2 supply as well as improved utilization of O_2 at the level of the muscle tissue; however, this increase was not seen during the exercise portion of the study as was hypothesized. Overall, the results did not show a strong effect of RIPC on exercise capacity for individuals with MS.

Mechanisms of Remote Ischemic Preconditioning

The exact mechanisms to explain the protective effects of RIPC are still being investigated; however, both neural and humoral physiological pathways are thought to be largely influential. It is evident that in order for RIPC to have its beneficial protective effects on tissues throughout the body the neural pathways must be intact, as observed when important nerve sections were occluded (femoral nerve and spinal cord) the effects of RIPC were abolished (Lim *et al.*, 2010; Donato *et al.*, 2013). Specifically regarding cardiovascular protection, the dorsal motor nucleus of the vagus nerve has been seen to be important for producing the protective effects of RIPC (Mastitskaya *et al.*, 2012). There is evidence that there is a strong combination between both the neural and humoral pathways as RIPC activates the peripheral nerves which then causes a release of protective humoral factors (Redington *et al.*, 2012; Mastitskaya *et al.*, 2016). The miRNA-144 effector molecule has been seen to decrease the size of infarction on occluded tissue and improve the functional recovery time following ischemia suggesting its major role in the process of RIPC (Hu *et al.*, 2014; Hess *et al.*, 2015). Additionally, nitric oxide (NO) has been observed to improve perfusion in RIPC (Hess DC, 2016), and administration of NO prior to preconditioning stimulates the effects of the conditioning while blocking NO synthesis contrarily inhibits the protective effects (Küntscher *et al.*, 2002). While the present study did not investigate the specific mechanisms of RIPC, the

previous findings help to explain the protective effects observed within prior studies and provide the basis for a clinical application.

Experimental Considerations

Due to the small sample size, the use of statistical analysis on the present findings was not appropriate to determine significance. Therefore, additional subjects of both the MS and control groups are needed in order to more accurately generalize the results to a clinical application. However, the trend of increased oxygen delivery and utilization within exercising skeletal muscle found within several subjects in this pilot study indicate that further investigation with a larger sample size would be worthwhile. In addition, results of previous studies that have found RIPC to help reduce inflammation and exercise tolerance among individuals with MS (Camara-Lemarroy *et al.,* 2018, Nair *et al.,* 2018) support the need to further investigate the role of RIPC on the exercise hyperemia response within this population.

In addition to the small sample size, there were several other limitations to this present study. The exercise stimulus of a rhythmic handgrip exercise at 10% MVC may have been too small of a load for the subjects to elicit large cardiovascular changes by the RIPC intervention. Similarly, while this study only included participants with MS that were low on the disability scale (EDSS <4.5) for consistency, the low level of impairment may not have elicited as large of a beneficial effect of the RIPC for those individuals.

Conclusions and Perspectives

Despite the lack of clear significant effects of RIPC within the present study, the trend of increased exercise hyperemia responses and oxygen utilization following RIPC of several participants are encouraging that upon further study among a greater population size, RIPC may serve as a safe and effective method to increase exercise capacity of individuals with MS. However, while the effects of RIPC on exercise hyperemia appear promising among the MS population, further research is needed in order to determine the clinical relevance and the most appropriate use of such an intervention within the widely diverse range of MS severity and symptoms.

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Figure Legends

Figure 1: Experimental Protocol

Following 20 minutes of quiet rest and instrumentation, subjects performed the rhythmic handgrip exercise. Resting FBF and SmO_2 measurements were taken during the baseline 4 minutes, followed by the exercise measurements taken during the 4 minutes of rhythmic handgrip exercise. In addition, as part of a secondary study/protocol within the same subjects (Goetz Thesis), after 20 minutes of rest, subjects also performed a measure of vascular function via reactive hyperemia. Following both measures, the RIPC took place in which 5 minutes of occlusion were followed by 5 minutes of perfusion repeated 4 times. The handgrip exercise and reactive hyperemia were both repeated following the RIPC, with 20 minutes of rest in between each measure.

Figure 2: Forearm Blood Flow

A. FBF values (open circles) are presented at the resting condition for each individual subject both before and after the RIPC intervention (lines represent the same subject across time points). The control subjects are represented by a black outline and the subjects with MS are represented with a red outline.

B. FBF values during the exercise condition are similarly represented for each individual subject by open circles and connected by lines for each subject before and after the RIPC intervention. The control subjects are shown by a black outline and the MS subjects are represented by a red outline.

Figure 3: Forearm Vascular Conductance

A. FVC values (open circles) are presented at rest for each individual subject pre- and post-RIPC with the lines connecting the individual subject's data points. Black outline indicates control subjects and red outline indicates subjects with MS.

B. FVC values during exercise are presented as open circles and connected by lines for each individual subject, with the black outline indicating control subjects and the red outline indicating MS subjects.

Figure 4: Muscle Oxygen Saturation

A. SmO_2 percentages are presented during the resting condition for each individual subject before and after the RIPC intervention, with the open circles indicating the subjects' SmO_2 value and the lines connecting the same subject's data across time points. The black outline indicates control subjects and the red outline indicates MS subjects.

B. SmO_2 percentages are similarly presented during the exercise condition as open circles with each individual's data connected by lines both before and after RIPC. Black outline indicates the control and the red outline indicates the participants with MS.

Tables and Figures
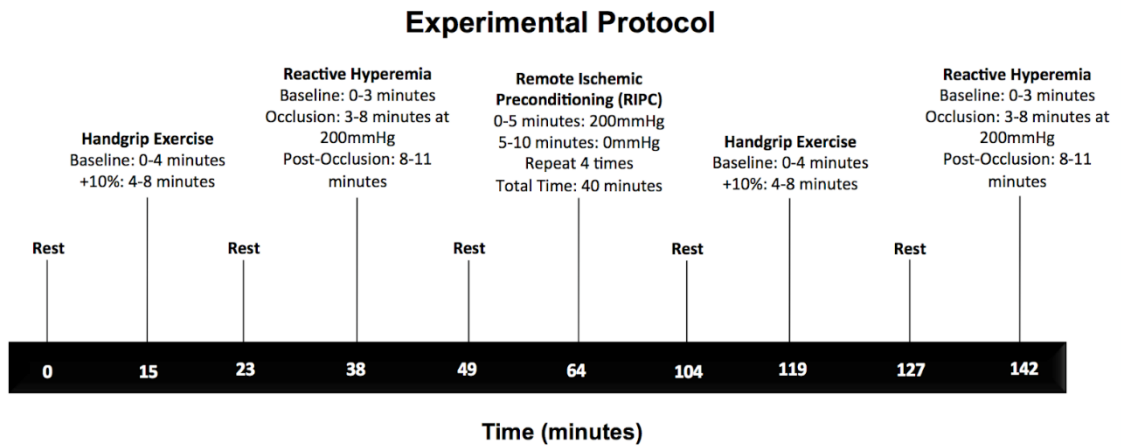
Figure 1: Experimental Protocol

Experimental Protocol

Handgrip Exercise
Baseline: 0-4 minutes
+10%: 4-8 minutes

Reactive Hyperemia
Baseline: 0-3 minutes
Occlusion: 3-8 minutes at 200mmHg
Post-Occlusion: 8-11 minutes

Remote Ischemic Preconditioning (RIPC)
0-5 minutes: 200mmHg
5-10 minutes: 0mmHg
Repeat 4 times
Total Time: 40 minutes

Handgrip Exercise
Baseline: 0-4 minutes
+10%: 4-8 minutes

Reactive Hyperemia
Baseline: 0-3 minutes
Occlusion: 3-8 minutes at 200mmHg
Post-Occlusion: 8-11 minutes

Rest   Rest   Rest   Rest   Rest

0   15   23   38   49   64   104   119   127   142

Time (minutes)

Table 1: Subject Characteristics

	Control (M=4, F=0)	MS (M=1, F=3)
Age (years)	35.75 ± 17.06	52.3 ± 7.4
Height (cm)	181.08 ± 1.63	165.7 ± 8.4
Weight (kg)	77.94 ± 2.98	62.3 ± 15
BMI (kg/m^2)	23.43 ± 0.95	22.7 ± 5.2
Body Fat %	18.075 ± 3.41	27.3 ± 9.5
EDSS	N/A	<4.5

Mean ± standard deviation, M=male, F=female

Table 2: System Hemodynamics (MAP, HR, Temp)

		Pre-RIPC		Post-RIPC	
		Rest	Exercise	Rest	Exercise
Control	HR (bpm)	51 ± 3	59 ± 5	57 ± 3	63 ± 3
	MAP (mmHg)	91 ± 3	101 ± 4	92 ± 2	93 ± 1
	Temp (°C)	29.4 ± 0.2	29.5 ± 0.1	31.9 ± 1.7	31.2 ± 1.4
MS	HR (bpm)	80 ± 3	86 ± 3	75 ± 3	82 ± 2
	MAP (mmHg)	94 ± 4	105 ± 6	89 ± 4	97 ± 10
	Temp (°C)	27.1 ± 1.2	27.3 ± 1.3	26.7 ± 0.8	26.6 ± 0.8

Mean ± standard error of means

Figure 2: Forearm Blood Flow

A. Rest

B. Exercise
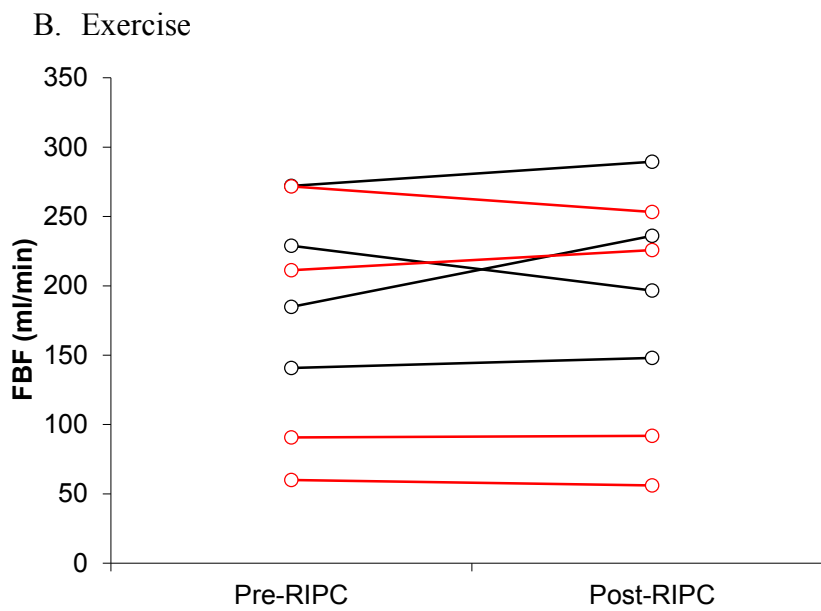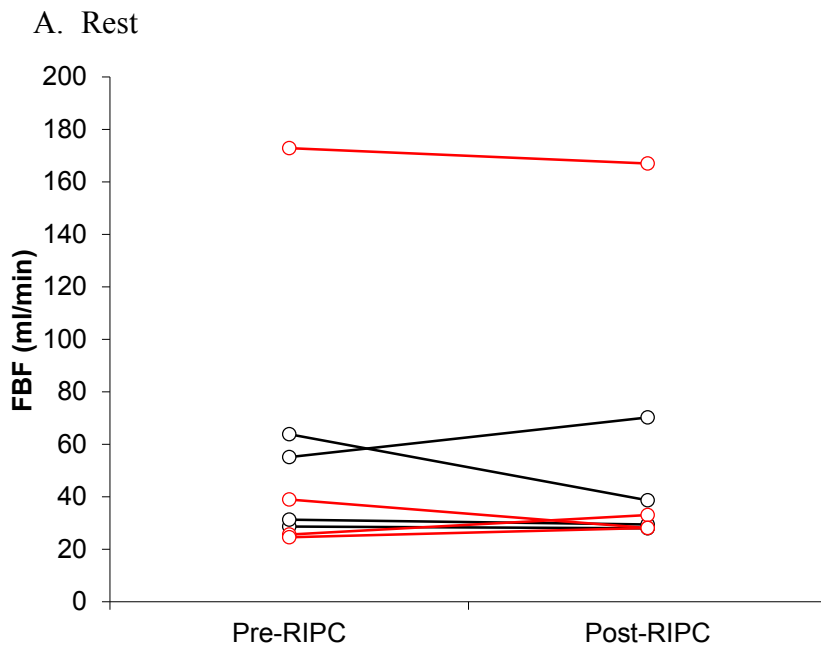
Control: ———

MS: ———

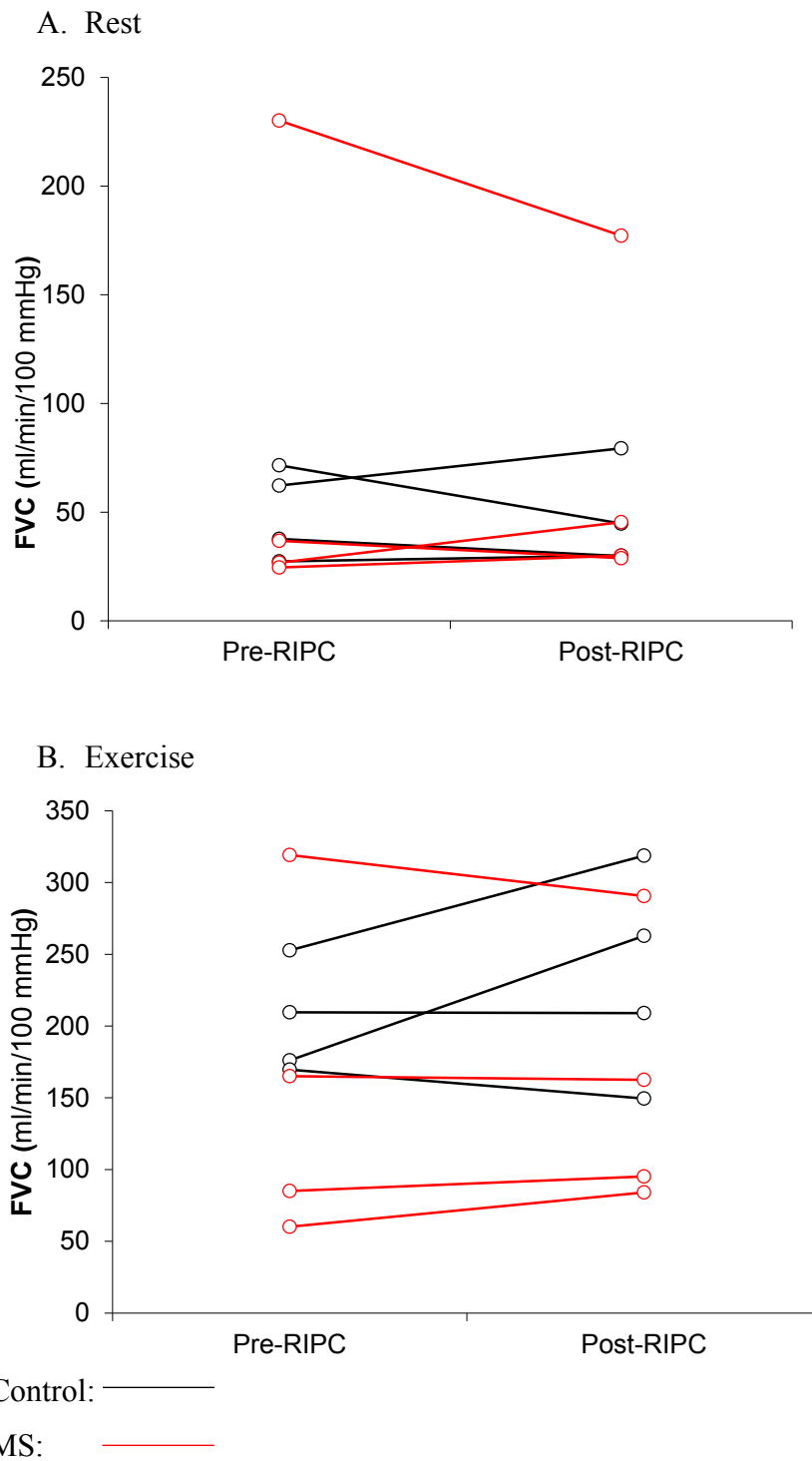Figure 3: Forearm Vascular Conductance

A. Rest

B. Exercise

Control: ———

MS: ———

Figure 4: Muscle Oxygen Saturation

A. Rest

B. Exercise

Control: ————

MS: ————